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# Pathogenic Exploitation of Fc Activity

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## INTRODUCTION

The phenomenon of antibody-dependent enhancement (ADE) underlies severe diseases across the entire spectrum of both microbes and vertebrates. Indeed, ADE could be classified as a fifth type of immunopathology (Table 19.1): Type I, IgE-mediated immediate hypersensitivity; Type II, IgG-mediated acute immune complex disease; Type III, IgG-mediated foreign antigen-complement-dependent immune complex disease; Type IV, cell-mediated immune and autoimmune diseases; and Type V, infectious immune complex enhancement of microbial infection in FcR-bearing cells.

Over the past four decades, different lines of scientific inquiry have coalesced to sharpen our understanding of antibody-mediated mechanisms that govern the severity of infections by a wide spectrum of microorganisms. Independent studies of host responses to acute and chronic human and animal infectious diseases have generated evidence that cross-linking of immune complexes with Fcγ receptors can increase intracellular infection and contribute to disease severity.<sup>1,2</sup> One of the earliest descriptions of this activity came from neutralization studies of Murray Valley encephalitis

virus (MVEV), in which exposure to virus in the presence of dilute avian antibodies resulted in greater clearance of chick embryo fibroblast monolayers than virus-only controls.<sup>3,4</sup> While follow-up research in the 1960s suggested that this phenomenon resulted from the stabilization of MVEV by antibodies,<sup>5</sup> a different explanation emerged when sequential infections in humans with dengue viruses (DENVs) were shown to produce severe disease (dengue hemorrhagic fever, DHF).<sup>6,7–10</sup>

During initial studies on ADE it had been assumed that increased virus output, which in some cases approached 100- to 1000-fold, resulted from extrinsic phenomena, such as the avid attachment to monocytes and macrophage and increased internalization of infectious immune complexes via interactions with FcγI and FcγIIa receptors as compared to virus alone, which was observed in numerous systems.<sup>9–15</sup> However, this effect of IgG opsonization can be complemented by a complex phenomenon involving suppression of innate cellular immunity. Unexpectedly, the incubation of Ross River virus (RRV) with diluted RRV antiserum resulted in enhanced infection in mouse macrophage cell lines and in primary human monocytes/macrophages via innate

**TABLE 19.1** The Five Types of Human Immunopathology

Type	Mechanism	Target
I	IgE antibodies	Antigens/mast cells
II	IgG antibodies	Autoantigens/tissue cells
III	IgG antibodies	Foreign antigens/PMNs
IV	Cell-mediated immunity	Tissue cells
"V"	IgG antibodies	Microbe/FcR-bearing cells

immune suppression involving reduced production of reactive nitrogen radicals via NOS2 and a downregulation of TNF- $\alpha$  and IFN- $\beta$  production through abolished interferon regulatory factor 1 (IRF-1) and nuclear factor- $\kappa$ B gene expression. Remarkably, a marked increase in IL-10 gene transcription and protein production was observed.<sup>16,17</sup> Surprisingly, ligation of Fc $\gamma$ R by zymosan–antibody complexes in the presence of RRV did not exhibit the same transcription patterns, indicating that features of the opsonized pathogen were involved in driving immune suppression.<sup>17</sup>

Similarly, many investigators showed that IgG bound to the surface of *Leishmania* amastigotes (the intracellular mammalian host-dwelling parasite stage) can induce the cytokine IL-10, which, in turn, can suppress the protective immune response to the parasite by downregulating inducible NO synthase (iNOS) and by inhibiting Th1 cell development and IFN- $\gamma$  production.<sup>18,19</sup> Hence, the IgG response, which is usually protective, is usurped by the parasite for its survival. When T-cell immunity fails, in the presence of abundant IgG anti-amastigote antibodies, infection starts on a lethal course. Thus, ADE is a complex phenomenon in which antibody-mediated interactions with Fc receptors may be involved not only in altering pathogen uptake into macrophage or monocytes but also in the cellular response to internalized pathogen.

## ADE IN VIRUSES

Antibody-enhanced viral infections require an initial adaptive immunological event, termed *sensitization*, which occurs in three settings: (1) Primary infections with naturally occurring heterotypic viruses of the same genera; (2) infection by viruses that create antigenic diversity by the rapid evolution of biologic or antigenic variants during the course of a chronic infection; and (3) immunizations that result in incomplete protective immunity (Table 19.2) (reviewed in Halstead<sup>11,20,22</sup> and Tirado and Yoon<sup>21</sup>). The ADE phenomenon has attracted wide interest in virology because many viruses replicate in macrophages *in vivo* and manifest antibody-enhanced infections/disease.<sup>21,23,24</sup>

### Group 1: Dengue

The dengue viruses (DENVs) are a group of four closely related members of the *Flavivirus* genus. DENVs 1 through 4 share 60 to 70% genetic homology and are inoculated by the bite of an infected *Aedes aegypti* mosquito. Initial infections with any of the four DENVs raise protective but type-specific antibodies in addition to cross-reactive but non-neutralizing antibodies that may enhance infection by a different DENV type.<sup>8,25–29</sup> Indeed, infection-enhancing antibodies are a risk factor for enhanced dengue disease.<sup>30</sup> That infants regularly acquire severe dengue disease during their first dengue infection when maternal polyclonal dengue antibodies circulate below protective levels is a unique illustration of the ADE phenomenon in human medicine.<sup>31–37</sup> In humans, secondary dengue infections follow a stereotypical course with severe outcomes, including shock or gastrointestinal hemorrhage, accompanying vascular collapse that results from capillary permeability occurring around the time of defervescence.<sup>38</sup> High levels of viremia early in disease and high levels of

**TABLE 19.2** Viruses Expressing ADE *In Vitro* or Producing Enhanced Disease

Viruses	<i>In vitro</i> ADE	<i>In vivo</i> Enhanced Disease	Refs.
<b>RNA Virus Group</b>			
Picornaviridae	Coxsackie B	+	Animal model Sauter and Hober <sup>121</sup>
Flaviviridae	Dengue	+	Halstead et al., <sup>7</sup>
	LDH	+	Stueckemann et al. <sup>122</sup>
Coronaviridae	Feline infectious peritonitis	+	Weiss and Scott, <sup>81</sup>
	PRRSV	+	Olsen, <sup>123</sup> Yoon et al. <sup>124</sup>
	Simian HF	+	
Retroviridae	HIV	+	Burke, <sup>125</sup> Mdurvwa et al., <sup>126</sup> Mealey et al. <sup>127</sup>
	Equine infectious anemia	+	
	Caprine arthritis	+	
<b>DNA Virus Group</b>			
Parvoviridae	Aleutian disease of mink	+	Porter et al., <sup>128</sup> Kanno et al., <sup>129</sup> Best and Bloom <sup>130</sup>
Asfarviridae	African swine fever		

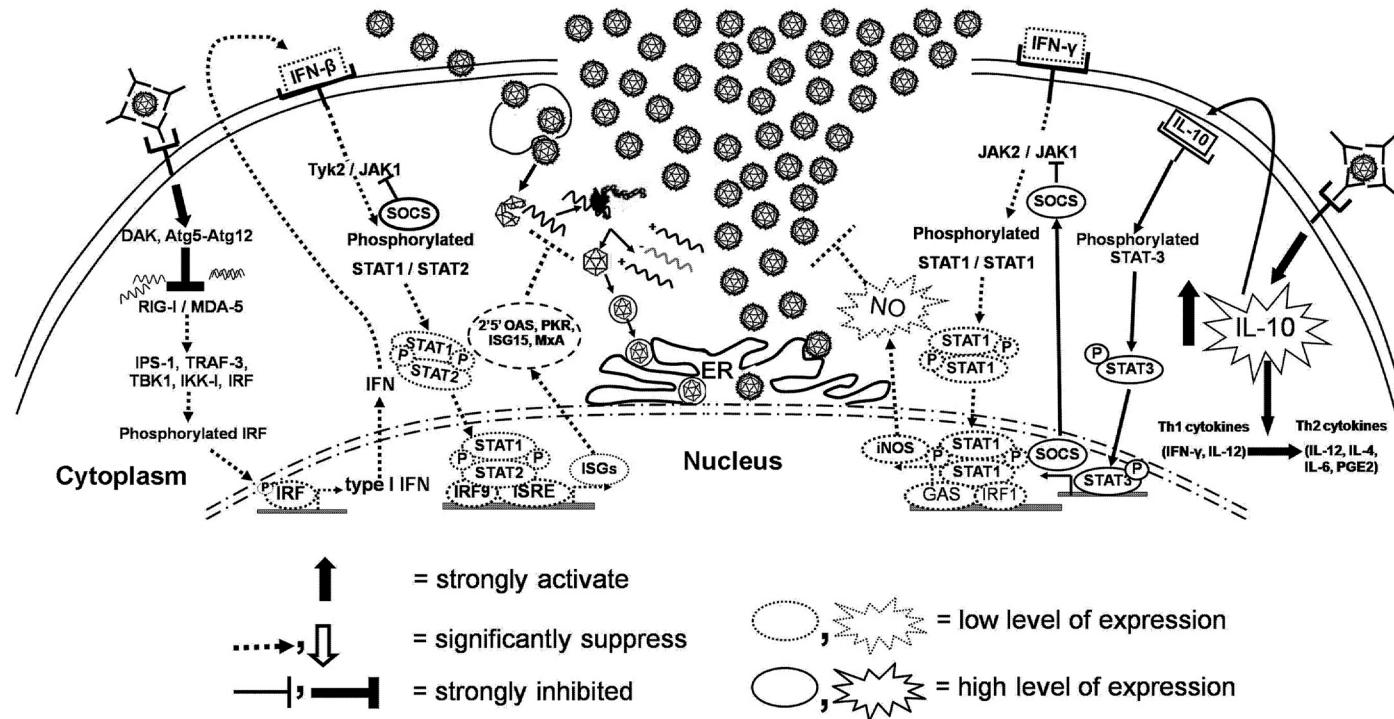
Note: Sensitization results are from sequential or chronic infection with multiple naturally occurring antigenic types.

proinflammatory and immunomodulatory cytokines including IL-10 late in disease are associated with severe outcome.<sup>39-41</sup>

*In vitro* studies indicate that the major requirement for ADE activity is a subneutralizing antibody concentration.<sup>22,42</sup> In practice, antibodies directed at surface epitopes not involved in virus entry efficiently produce ADE.<sup>43</sup> In studies of ADE in the THP-1 cell model (human monocytic Fcγ receptor-bearing continuous cell line) intracellular DENV production was increased as a result of idiosyncratic Fcγ receptor signaling.<sup>44</sup> When immune complexes ligate FcγRI and FcγRIIA, at least two types of suppression pathways are expressed (Figure 19.1). Collectively, these pathways downregulate antiviral responses in ADE-infected target cells. As a result, ADE-infected THP-1 cells secreted reduced levels of type-I IFN and at the same time suppressed the

transcription and translation of IL-12, IFN-γ and TNF-α, facilitating expression and synthesis of the antiinflammatory cytokines. ADE infection also suppressed the innate anti-DENV mediator, nitric oxide radicals, by disrupting the transcription of the iNOS gene transcription factor, IRF-1,<sup>44</sup> believed to be mediated by IL-10.<sup>45</sup> It can be concluded that *in vitro* ADE infection not only facilitates viral entry but also modifies innate and adaptive intracellular antiviral mechanisms resulting in enhanced DENV replication.

Critically, the same responses are observed *in vivo*. Genome-wide transcriptomes from peripheral blood mononuclear cells (PBMCs) collected during the acute phase from children with dengue fever (DF) or dengue hemorrhagic fever (DHF) indicated that patients with DHF had decreased levels of NO, reduced IFN transcript, and increased IL-10 blood levels compared to patients with milder illness.<sup>46</sup> In other



**FIGURE 19.1** The two-loop model of ADE. Ligation of Fc $\gamma$ R by infectious DENV-subneutralizing antibody complex induces suppression of innate immune responses: (1) By upregulation of negative regulators of pathogen pattern recognition, DAK, Atg5-Atg12, SARM, and TANK, that subsequently abolish expression of RLR and TLRs and its signaling pathway, resulting in decreased type-1 interferon and proinflammatory cytokines production. These serve to suppress interferon-mediated antiviral responses. (2) By early activation of IL-10, which potently activates the SOCS system suppressing the JAK/STAT signaling pathway and, in turn, the interferon-signaling pathway. IL-10 is known to promote type Th2 cytokines response, resulting in Th2-type cytokine biasing. These two loops of suppression switch off intracellular antiviral responses in DENV-ADE-infected cells, resulting in the production of high numbers of infectious virions. (From Ulbol, S. and Halstead, S.B., Clin. Vaccine Immunol., 17(12), 1829–1835, 2010. With permission.)

studies, during the acute stage of severe disease increased production of IL-10 and downregulation of multiple IFN regulatory genes were noted.<sup>47–49</sup> The protective role of IFN in moderating dengue infection has been demonstrated in a mouse model and suggested for humans with DF.<sup>50–52</sup> The precise role of immune complex-elicited IL-10 production on the clinical evolution of severe dengue infections is not well understood but may be responsible for the observed Th1 to Th2 shift observed in DHF.<sup>53</sup>

Results obtained by studying ADE in continuous human FcR-bearing cells are subject to a critical caveat: Are responses observed faithful to those occurring *in vivo*? When dengue ADE was studied in four different primary human myeloid cells derived from the same peripheral blood leukocyte (PBL) donors, viral infection and cytokine responses differed significantly.<sup>54</sup> Human monocytes, activated macrophages, and mature dendritic cells (DCs) support ADE, while immature DCs did not. Infection of macrophages by DENV 2 alone or as fully neutralized immune complexes stimulated high levels of  $\alpha$  and  $\beta$  IFN, and these were downmodulated under ADE conditions and replaced by secretion of IL-6 and TNF- $\alpha$ . Type I IFNs were neither produced nor suppressed by ADE infection of monocytes with DENV 2.<sup>54,55</sup> However, during ADE infection of primary monocytes, IL-10 synthesis peaked at the same serum dilution that produced peak virus yield.<sup>54</sup> The precise mechanisms fueling enhanced production of dengue virus in monocytes and macrophages require further study, as neither the production of IL-10 nor suppression of type I interferon is critical to the ADE phenomenon. There is some evidence that the degree of antibody opsonization may impact Fc $\gamma$ R interactions, resulting in differential processing of surface-bound virus. In particular, in the THP-1 model, large immune complexes have been found to stimulate Fc $\gamma$ IIB, resulting in inhibition of cellular uptake.<sup>56</sup>

In considering the contribution of FcR-mediated phenomena during the course of

acute infectious diseases it is important to note the differing kinetics of microbial invasion and response to infection. Infections in vertebrates can be divided into *afferent* and *efferent* events. *Afferent* events are those that relate to the invasion, propagation, and survival of infecting organisms. *Efferent* events are those contributing to reduce, retard, and eliminate infecting organisms. For those infections that produce disease, afferent events successfully predominate during the early stages, while efferent events may culminate in elimination of invading organism and cure. In the ADE phenomenon, IgG antibodies residual from a previous dengue infection, whether actively produced or passively acquired, act as powerful afferent events. IgM and, in the case of secondary dengue infections, IgG dengue antibodies play important roles in the efferent events of dengue infections, some of which include clearing extracellular virus, activating complement, and mediating ADCC.

In dengue-endemic countries, second heterotypic dengue infections are responsible for up to 95% of DHF, while primary dengue infections in infants account for the remainder.<sup>57,58</sup> The *in vitro* ADE literature makes it clear that antibodies to virtually any flavivirus can enhance dengue virus infection in FcR-bearing cells.<sup>59</sup> In Southeast Asia, the dengue viruses co-circulate with Japanese encephalitis (JE); in India, with JE and West Nile; in Pakistan, with West Nile; in Australia, with the Kunjin strain of West Nile; and, in most of the American region, yellow fever or use of yellow fever 17D vaccine. A direct examination of enhancement of DENV 2 infections in a human monocytic cell line by antibodies raised in humans who had experienced apparent wild-type JE infections was negative.<sup>60</sup> However, this study screened for ADE at just a single high dilution of serum. There have been no reports of enhancement of dengue infections by prior West Nile or yellow fever infections (or vaccination); however, recently it was observed that children in Thailand who had either had wild-type JE infections or were

immunized with killed JE vaccine experienced a mild overt dengue disease at a higher frequency than when the same dengue virus infected susceptible individuals.<sup>61</sup> Not only did dengue infections in JE-immunes increase the frequency of mild overt dengue disease, but the ensuing illness lasted two times longer than dengue illnesses in susceptibles. Prior to this observation it was thought that JE vaccination actually reduced the severity of DHF accompanying a second dengue infection.<sup>62</sup>

Second DENV infections can occur in 12 combinations, at least 10 of which have been documented to result in hospitalized disease.<sup>58</sup> Human experimentation has established that there is a DENV infection refractory period of three months following an initial DENV infection. As presented briefly above, shortly after a primary DENV infection abundant heterotypic antibodies may form large immune aggregates that are neutralized in solution but not eliminated by standard phagocytic clearance by macrophages.<sup>56</sup> After the refractory period, second dengue infections occur and some are expressed as clinical illness. Varying amounts of heterotypic neutralizing antibodies are raised following a primary dengue infection. The natural histories of antibody responses following infection by DENVs 1, 2, 3, or 4 including heterotypic antibodies are essentially unknown. Heterotypic antibodies are critically important in modulation of a second DENV infection.<sup>30</sup> Low dilutions of preinfection sera from children who had unapparent secondary DENV 2 infections almost invariably reduced or neutralized DENV 2 in primary human monocytes, while preinfection sera from children who developed an illness requiring hospitalization had few or no detectable heterotypic neutralizing antibodies.<sup>30</sup> This observation suggests that low levels of heterotypic neutralizing antibodies (most were anti-DENV 1) did not prevent but downregulated DENV 2 clinical responses. Around one-fifth of monotypically dengue-immune schoolchildren lacked heterotypic DENV 2 antibodies and

developed DHF when infected by DENV 2. This is virtually the same ratio for the occurrence of dengue shock syndrome (DSS) during sequential DENV 1 then DENV 2 in Rayong, Thailand.<sup>63</sup>

The sequence of infection may be highly determinative of disease severity. Only secondary DENV 2 infections were pathogenic in the 1980 cohort study at Rayong, Thailand.<sup>63</sup> The specific infection sequences associated with DSS cases were known from virus isolations in acute-phase sera and antibodies in pre-illness sera or by applying the original antigenic sin phenomenon to paired sera.<sup>64</sup> Although secondary DENV 1 infections were most common that year, DSS occurred only during secondary DENV 2 infections. Burmese workers came to a similar conclusion in their 1984 to 1988 longitudinal seroepidemiological study in Yangon, Myanmar.<sup>65</sup> By contrast, in an Indonesian study, DSS was associated with sequences ending in DENV 1, 3, and 4, but not DENV 2, even though secondary DENV 2 infections were common.<sup>66</sup> DENV 3 was associated with an outbreak of DHF/DSS on Tahiti in a population that had prior infection experience with DENV 1 and DENV 2.<sup>67</sup> Also on Tahiti in 2001, DENV 1 infections resulted in DHF/DSS in 4–11 year-old children who were immune to DENV 2.<sup>68</sup> Over time the pathogenicity of established DENV infection sequences may change. Notably, there was a rapid increase in pathogenic expression of secondary DENV 2 infections (in DENV 1-immunes) month to month in Cuba.<sup>69</sup> Complete and partial sequencing of DENV 2 viruses isolated from the beginning to the end of this outbreak identified no structural genetic mutations but consistent amino acid changes at position 69 in the NS1 protein.<sup>70,71</sup> A more complex study in Managua, Nicaragua, attributed year-to-year increases in disease pathogenic expression to a combination of genetic changes in DENV 2 and a waning DENV 1 immunity in the affected children.<sup>72</sup> Essentially none of these field observations has been subjected to study using cultures of primary human myeloid cells.

From the work cited above it must be concluded that ADE studies should not be performed on continuous human myeloid cells but instead on characterized primary human myeloid cells. To date, all research has been carried out using DENV 2. Studies of ADE should be extended as quickly as possible to the complete range of dengue viruses using type-specific polyclonal human antibodies rather than mouse or even human monoclonal antibodies. More observations on human dengue illnesses are needed to better understand the ADE phenomenon. In this context, understanding DHF in infants that accompanies primary DENV infections acquired during the latter half of the first year of life is important and has attracted recent research interest.<sup>33,34</sup> This unique clinical phenomenon illustrates not only the role played by dengue antibodies in modulating disease expression but also their bifurcated role—protection for several months after birth at high concentrations and enhancing infections some months later at sub-neutralizing concentrations. The contribution of immature DENV particles to ADE is of interest. Immature DEN virions are not infectious for human myeloid cells, but in the presence of enhancing dengue antibodies ADE infection occurs readily.<sup>43,73</sup> It has been surmised that immature DENV are released into circulation during human infections as antibodies to prM (immature DENV antigen) and are frequently observed.<sup>43</sup>

Note has been made above of the possible role of different Fc<sub>y</sub> receptors in mediating ADE in macrophages in leishmania mouse models and the interesting observation suggesting that ligation of Fc<sub>y</sub>RIIB may affect the fate of large immune complexes during the immune clearance phase of dengue infections. The real research challenge is to document the critical Fc<sub>y</sub> receptors that are operative during human or veterinary clinical microbial disease enhancement episodes. But, more important will be to study the specific outcome of ADE infection by different wild-type dengue viruses—antibody

combinations including appropriate genotype variants. The possibility that differing combinations of DENV and dengue antibodies (or, for that matter, antibodies to other flaviviruses) have uniquely different effects on monocyte/macrophage innate immunity requires careful study.

It is widely held that the process of eliminating dengue virus-infected cells generates a cascade of chemokines and cytokines that contribute to the pathophysiology of dengue disease syndromes. This has been termed “a perfect cytokine storm.”<sup>74</sup> Given the evidence of increased infected cell mass in severe dengue infections, the cytokines generated by virus infection and by interactions between virus-infected cells and the host immune response would appear to be quantitatively proportional to viral load and not exaggerated or “abnormal.” Because dengue is not a cytophilic virus, cellular infection proceeds until the infected cell is eliminated. A wide misconception is that peak viremia, which occurs early in infection, represents peak “viral load.” In fact, the quantity of virus in blood during the course of infection only describes the kinetics of extracellular virus clearance. Using the sensitive technique of intrathoracic inoculation of *Toxorrhynchites splendens* mosquitoes,<sup>40,75</sup> it was observed that dengue virus could be detected in nearly all plasma samples collected within 72 hours of the onset of fever. The frequency of virus isolation declined rapidly and virus was usually not present at defervescence.<sup>76</sup> Viremia was of longer duration in individuals experiencing primary dengue virus infections than in those experiencing secondary dengue virus infections, and the slope of the decline in virus titer was greater in individuals with DHF than in those with DF.<sup>40</sup> The most important mechanism of extracellular virus clearance is thought to be IgM and IgG dengue antibodies, although T lymphocytes and antibody-dependent cell killing may contribute by attacking and destroying virus-infected cells. As discussed above, it is likely that elimination

of dengue virus-infected cells continues well after onset of antibody production resulting in peak cellular infection (viral load) at around the time of defervescence and cellular infection is not eliminated until well after end of viremia. Of interest, a major effort failed to detect circulating CD8+ T cells during the late acute-illness phase of DHF patients.<sup>77</sup> This suggested to the authors that CD8+ T cells might be located only in the tissues where dengue virus replication had occurred. Alternatively, as in a Japanese encephalitis model, the antibody component of the adaptive immune response may play a much more important role in terminating dengue infections than heretofore thought.<sup>78</sup>

## Groups 2 and 3

Feline infectious peritonitis virus (FIPV) is an example of Group 2/3 virus immunopathology

involving ADE (Table 19.2). Feline coronaviruses circulate as two forms: Feline enteric coronavirus (FECV) is a pathogen of minor significance; however, a spontaneous mutation of this virus yields FIPV, which is capable of replicating in peritoneal macrophages and producing peritonitis and occasionally FIP, a fatal Arthus-like pyogranulomatous disease in kittens and cats. ADE has been incriminated as a disease-enhancing factor.<sup>79–81</sup> That antibodies are pathogenic is evidenced by observations that kittens who have acquired passive maternal FIPV antibodies develop a more rapid and fulminant disease following challenge with FIPV than do seronegatives.<sup>81</sup> Disease enhancement has been demonstrated in cats that were infected in the presence of vaccine-derived humoral immunity directed against the spike protein of FIPV (Table 19.3).<sup>82</sup> Similarly, cats immunized with a recombinant vaccinia virus

**TABLE 19.3** Enhanced Viral Diseases Resulting from Sensitization by Administration of Vaccines

	Viruses	In vitro ADE	In vivo Enhanced Disease	Refs.
<b>RNA Virus Group</b>				
Orthomyxoviridae	Influenza A	+	+ (mouse model)	Webster and Askonas <sup>131</sup>
Paramyxoviridae	RSV	+	+	Chanock and Parrott, <sup>132</sup> Fulginiti et al. <sup>133</sup>
	Measles	+	+	
Rhabdoviridae	Rabies	+	+ (accelerated disease onset)	King et al., <sup>134</sup> Prabhakar and Nathanson <sup>135</sup>
Coronaviridae	Feline infectious peritonitis	+	+	Tirado and Yoon, <sup>21</sup> Weiss and Scott, <sup>81</sup> Yoon et al. <sup>124</sup>
	PRRSV	+	+	
	Simian HF	+	+	
Retroviridae	HIV	+	?	Mdurvwa et al., <sup>126</sup> Mealey et al., <sup>127</sup> Burke <sup>125</sup>
	Equine infectious anemia	+	+	
	Caprine arthritis	+	+	
<b>DNA Virus Group</b>				
Parvoviridae	Aleutian disease of mink	+	+	Porter et al. <sup>128</sup>

expressing the spike protein of FIPV died earlier than control animals.<sup>82</sup> In adult cats, FIPV develops during chronic infections with feline coronaviruses after FECV mutates to FIPV, gaining macrophage tropism.<sup>83</sup> As antibody responses are mounted to FIPV, infection and disease severity are enhanced.<sup>79</sup> In summary, antibody responses to FIPV occurring during the course of infection, passive transfer of antibodies from natural infections, and immunization with killed or recombinant vaccines all have led to enhanced FIPV disease. Enhanced disease severity has been attributed largely to the presence of non-neutralizing antibodies.

## ADE IN PROTOZOAN PARASITES OF MACROPHAGES: LEISHMANIA

Leishmaniasis is caused by protozoan parasites of the genus *Leishmania* which are transmitted by the bite of certain species of sandfly. Human infection is caused by about 21 of 30 *Leishmania* species that infect mammals. The disease exists in two major forms, cutaneous and visceral leishmaniasis. Visceral leishmaniasis (VL) is the most severe form of leishmaniasis and the second largest parasitic killer in the world after malaria, responsible for an estimated 500,000 cases each year worldwide. *Leishmania* are transmitted by sandflies as promastigotes, motile forms that parasitize macrophages and spread within hosts as amastigotes, which are obligate parasites of macrophages. In human hosts the responses to infection by *Leishmania* vary with species and the patient's immune reaction. Patients whose lymphocytes produce amounts of IFN- $\gamma$  from Th1-type T cells often recover from cutaneous infection on their own and after recovery are immune to reinfection. Patients infected with visceral forms of the parasite make high levels of anti-parasite antibody that does not contribute to host defense. Without treatment, these patients are likely to succumb to VL. Mouse models exist for the

study of cutaneous and VL. For example, normal BALB/c mice are susceptible to this disease and develop progressive non-healing lesions with numerous intracellular parasites within macrophages. Despite the presence of high levels of anti-parasite antibody, mimicking human VL, these parasites often disseminate to the liver, spleen, and bone marrow. *In vitro* models are also available—promastigote or amastigote infections in cultures of bone marrow or peritoneal macrophages from mice or in differentiated peripheral blood human monocytes.

During the 1980s and 1990s a number of mammalian cytokines were discovered; among them was mouse cytokine synthesis inhibitory factor produced by Th2 cells, later renamed IL-10.<sup>84,85</sup> IL-10 is a type II cytokine and the “founding” member of a family of cytokines that includes IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, and IL-29.<sup>86</sup> All of these cytokines have similar intron-exon genomic organization, bind to receptors with similar structures and in some cases shared components, and all activate Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathways. IL-10 is capable of inhibiting synthesis of pro-inflammatory cytokines such as IFN- $\gamma$ , IL-2, IL-3, IL-12, TNF- $\alpha$ , and GM-CSF. IL-10 also displays a potent ability to suppress the antigen presentation capacity of antigen-presenting cells. There are, however, some settings in which IL-10 is not immunosuppressive. IL-10 can enhance NK and B-cell survival and B-cell antibody production.<sup>87</sup> IL-10 is made by macrophages, and a variety of T cells, including T reg, Th1, Th2, and Th17.<sup>88</sup>

IL-10 plays important roles in regulating immune responses and it can also increase host susceptibility to intracellular infections. In the mouse model of cutaneous leishmaniasis susceptibility of *Leishmania major* is associated with Th2 responses. CD4 $^{+}$  T cells from susceptible BALB/c mice produced IL-4 and IL-10 when infected with *L. major*, while CD4 $^{+}$  T cells from resistant C57BL/6 mice expressed IFN- $\gamma$  and

IL-2.<sup>89</sup> The transient depletion of CD4<sup>+</sup> cells or the *in vivo* neutralization of IL-4 in BALB/c mice promoted the killing of intracellular parasites and healing of leishmanial lesions. IL-4 was not the only determinant of susceptibility, however, because susceptible BALB/c mice lacking IL-10 were fully resistant to infection.<sup>18</sup> Furthermore, in humans where the Th1/Th2 dichotomy is not as pronounced, IL-10 appears to be a major inducer of susceptibility. In humans with visceral leishmaniasis (VL), IL-10 levels in plasma directly correlate with disease severity.<sup>90</sup> IL-10 was identified in lymph nodes taken from patients with VL.<sup>91</sup> PBMCs from acute visceral leishmaniasis responded to stimulation with leishmania lysate by producing IL-10 mRNA. IL-10, when added to PBMCs, suppressed production of IFN-γ and IL-2 but after treatment with anti-IL-10 PBMCs from patients with acute VL demonstrated a marked increase in proliferative response to leishmania lysate. *In vitro* studies of *Leishmania*-infected peritoneal and bone marrow macrophages demonstrated that the intracellular killing of this organism by classically activated macrophages could be inhibited by the administration of exogenous IL-10<sup>92,93</sup> or by endogenous macrophage production of IL-10.<sup>92</sup>

Greater clarity was achieved when the reciprocal actions of IL-12 and IL-10 were recognized. The complementary cytokine IL-12 acts directly on CD4<sup>+</sup> T cells to enhance priming for IFN-γ production and reverse IL-4 priming.<sup>94</sup> The most potent cytokine for the induction of leishmanicidal activity in macrophages is IFN-γ.<sup>95</sup> The sustained production of IFN-γ in response to infection is commonly associated with the development of specific Th1 T-cell responses. Administration of IL-12 increased IFN-γ production and reduced the severity of *Leishmania major* infections in mice and potentiated vaccine-derived immunity and depressed IL-10 production.<sup>96</sup> However, exposure of macrophages from susceptible mice to opsonized *Leishmania* promastigotes suppressed the expression of IL-12.<sup>97</sup>

The importance of macrophage receptors in the generation of cytokines in *Leishmania*-infected macrophages was recognized when IL-12 production in BALB/c mouse bone marrow macrophages in response to LPS was suppressed after ligation of FcR, complement or scavenger receptors.<sup>98</sup> Both mRNA synthesis and protein secretion were diminished to near-undetectable levels following receptor ligation. Suppression was specific to IL-12 since TNF-α production was not inhibited. Also, the ligation of mouse FcγR with immune complexes was shown to enhance the production of IL-10.<sup>99</sup> Stimulation of mouse bone marrow macrophages by LPS resulted in some IL-10 production, but the addition of RBC opsonized with IgG antibodies dramatically enhanced IL-10 production. Immune complexes not only induce activated macrophages to produce IL-10 but also induce both macrophages and dendritic cells to switch off their production of IL-12.<sup>98,100</sup>

The modulation of IL-10 and IL-12 in macrophages results from different mechanisms.<sup>101</sup> Whereas the abrogation of IL-12 biosynthesis was a property shared by ligation of several macrophage receptors, the induction of IL-10 was specific to FcγRs. Additional evidence for the role of IL-10 in supporting chronicity of infection was obtained when normal BALB/c mice developed progressive non-healing lesions with numerous *Leishmania major* parasites while IL-10<sup>-/-</sup> BALB/c mice controlled disease progression and had relatively small lesions with 1000-fold fewer parasites at the fifth week of infection.<sup>18</sup> Furthermore, in established *L. donovani* visceral infection in normal mice, anti-IL-10 or anti-IL-10 receptor monoclonal antibody (mAb) treatment successfully induced intracellular parasite killing within liver macrophages.<sup>102</sup> These and other studies showed that amastigotes of *Leishmania* exploit an unusual and unexpected virulence factor, host IgG. When the surface of *Leishmania* amastigotes is coated with IgG, the resultant immune complexes allow them to ligate

Fc $\gamma$  receptors on inflammatory macrophages, preferentially inducing the production of high amounts of IL-10. The IL-10 induction by IgG-amastigotes did not occur in macrophages from mice lacking the common gamma chain that signals through Fc $\gamma$ Rs 1, 3, and 4, indicating that one or all three of these receptors were involved. Subsequent studies using defined immune complexes demonstrated that all three of the Fc $\gamma$ Rs that signal through gamma were capable of signaling for IL-10 production in macrophages.<sup>103</sup> The implication from these studies is that in some settings IgG itself biases the immune response toward a Th2-type response. Indeed, for some species of *Leishmania*, chronicity of infection requires that amastigotes be coated with IgG.<sup>104</sup> This phenomenon is now very well established.<sup>93,105,106</sup>

That IL-10 induction by ligation of Fc $\gamma$ R was a generic process was demonstrated with a non-microbial antigen.<sup>107</sup> Lipopolysaccharide-treated BALB/c mouse macrophages when exposed to ovalbumin (OVA) alone developed T-cell responses driven to Th-1 and characterized by the production of IFN- $\gamma$ . When the same antigen was complexed with IgG anti-OVA, T-cell responses were driven to Th2 and produced IL-4. This Th2-like phenotype was stable and was retained when the T cells were subsequently restimulated under non-biasing conditions. Mice vaccinated with IgG-opsonized OVA made high levels of IgG Abs of the IgG<sub>1</sub> isotype. The T-cell biasing and its reversal via Fc $\gamma$ R ligation was also observed *in vivo*. Using macrophages from gene knockout mice, the production of IFN- $\gamma$  and IL-4 by T cells was shown to be controlled by the macrophage cytokines, IL-12 and IL-10, respectively. These and other studies demonstrate that the ligation of Fc $\gamma$ R on activated macrophages reverses Th1 biasing that accompanies innate immune responses to microbial products.

Importantly, in patients with VL, high levels of anti-leishmanial antibodies correlate with peak parasitemia and with negative DTH

responses.<sup>93</sup> Successful treatment of leishmaniasis with amphotericin resulted in decreased antibody titers and a restoration of DTH responses. Earlier observations identified polyclonal B-cell activation and high levels of immune complexes as well as rheumatoid factor in patients with VL.<sup>108</sup> Humans infected with *Leishmania donovani* had higher frequencies of rheumatoid arthritis.<sup>109</sup> In experimental models of VL, infected hamsters develop immune complex glomerulonephritis. These observations have been widely confirmed in mouse models. In addition to *Leishmania donovoni* and *L. major*, humoral immune responses against *L. mexicana* were not effective at killing organisms hiding in parasitophorous vacuoles because host IgG-coated amastigotes generated immunosuppressive IL-10 responses by infected macrophages.<sup>19,110</sup> A similar observation, although perhaps occurring by a different mechanism, may be involved in *L. amazonensis*-infected JhD mice in which infections were minimized in the absence of B cells or antibodies. When these immune elements were restored leishmania lesions progressed by a process thought to involve CD4 $^{+}$  T cells.<sup>111</sup>

How do antibody-coated amastigotes result in the production of IL-10 by macrophages? Ligation of macrophage Fc $\gamma$ R produces a rapid and enhanced activation of two MAPKs, ERK and p38.<sup>112</sup> The activation of ERK leads to the phosphorylation of serine 10 on histone H3 at the *IL-10* gene, making the promoter more accessible to transcription factors generated in response to p38 activation.<sup>95</sup> Activation of both MAPKs was required for IL-10 synthesis. In addition to ERK activation, an inflammatory stimulus, such as low-molecular-weight hyaluronic acid from the extracellular matrix, must also be present. The combination of these two signals resulted in the superinduction of IL-10.<sup>113</sup> Macrophages lacking Fc $\gamma$ R, or macrophages treated with an inhibitor of spleen tyrosine kinase that is activated following Fc $\gamma$ R ligation, failed to activate ERK and consequently failed to produce IL-10 following infection with *Leishmania* amastigotes.

Recently, however, it has been observed that IgG<sub>1</sub> and IgG<sub>2a/c</sub> induce IL-10 from mouse macrophages *in vitro* equally well but through different Fc $\gamma$ R subtypes: IgG<sub>1</sub> through Fc $\gamma$ RIII, and IgG<sub>2a/c</sub> through Fc $\gamma$ RI primarily but also through Fc $\gamma$ RIII. In sharp contrast, mice lacking IgG<sub>1</sub> develop earlier and stronger IgG<sub>2a/c</sub>, IgG<sub>3</sub>, and IgM responses to *Leishmania mexicana* infection and yet are more resistant to the infection.<sup>114</sup> Thus, IgG<sub>1</sub>, but not IgG<sub>2a/c</sub> or IgG<sub>3</sub>, is pathogenic *in vivo*, in agreement with prior studies indicating that Fc $\gamma$ RIII is required for chronic disease. This calls into question the assumption that mouse macrophages, which should secrete IL-10 in response to both IgG<sub>1</sub> and IgG<sub>2a/c</sub> immune complexes, are the most important source of IL-10 generated by IgG-Fc $\gamma$ R engagement in *L. mexicana* infection.

## ADE IN OTHER INTRACELLULAR PARASITES AND BACTERIA

The possibility that FcR-mediated enhancement of disease occurs across a broad range of microorganisms has received little attention by research communities. The possibility that ADE contributes to severity or chronicity of disease exists because a large number of bacteria replicate partially or solely in human macrophages. Larger microorganisms that infect macrophages, “professional intracellular pathogens,” often produce chronic infections. Indeed, one of the criteria of successful parasitism by microorganisms that produce systemic infections may be the ability to evade macrophage microbicidal mechanisms. These defensive mechanisms have been subject to intensive scrutiny.<sup>115</sup> Both chronic infections and an antigenic relatedness between microorganisms may contribute to the formation of pathogenic IgG immune complexes controlling microbial survival. Infections that may be analogous to the *Leishmania* model include

*Mycobacterium tuberculosis* (Mtb), *Mycobacterium leprae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Brucella* spp., *Salmonella* spp., *Shigella* spp., *Coxiella burnetii*, *Anaplasma phagocytophilum*, and *Ehrlichia chaffeensis*, as well as another protozoan, *Toxoplasma gondii*, and fungi (e.g., *Histoplasma capsulatum*).

The role of Fc receptors or immune complexes in mediating immunopathology has not been studied exhaustively for most of the organisms on the above list; however, interesting findings have been made in tuberculosis. Although studies on resistance to tuberculosis have centered on T-cell immunity, in a recent experiment C57BL/6 mice deficient in inhibitory Fc $\gamma$ RIIB showed improved bacterial control and diminished pathology at 30 but not 20 days following aerosol challenge with *Mycobacterium tuberculosis*.<sup>116</sup> Enhanced production of IL-12p40 was observed in Fc $\gamma$ RIIB<sup>-/-</sup> mice. IL-12 and IL-23 share the common subunit IL-12p40 and both promote the polarization of naive CD4<sup>+</sup> T cells into Th1 effectors.<sup>117</sup> Treatment of human macrophages with exogenous IL-12 combined with blockade of IL-27 reduced the burden of mycobacterial infection.<sup>118</sup> These infections were characterized by enhanced protective IFN- $\gamma$  responses that coincided with heightened activation of macrophages. In humans, elevated levels of IL-10 correlate with the suppression of host defenses and exacerbation of infection.<sup>119</sup> In a model of reactivation tuberculosis the presence of macrophage-derived IL-10 in the lungs of infected transgenic mice permitted Th1 cells to efficiently express effector functions and secrete sufficient IFN- $\gamma$  to induce classical macrophage activation characterized by expression of NOS2 and LRG-47.<sup>120</sup> However, mycobacteria survived and successfully proliferated within mouse macrophages with IL-10 production under control of a human CD68 promoter. Macrophage-derived IL-10 appears to override IFN- $\gamma$ -dependent classical macrophage activation and other effector mechanisms against Mtb by inducing an alternatively activated

phenotype. To date, an explicit contribution of Mtb–IgG antibody complexes toward modulation of infections in model systems has not been reported.

## CONCLUDING THOUGHTS

Successful pathogens are armed with an array of weapons, including penetration and cell attachment factors and a galaxy of defenses against innate immune responses. Much is known and there is still much to learn about the offensive attributes of microbial pathogens. However, because of the ADE phenomena, antibodies very likely complement and contribute a generic and thus far poorly explored afferent disease mechanism for many infectious diseases in addition to the examples cited here. Cognizance of the need to balance protective and potentially harmful antibody activities remains an important concern in vaccine development.

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